Olfactometric Evaluation of Spatial Repellents for *Aedes aegypti*

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J. Med. Entomol. 40(4): 463–467 (2003)

ABSTRACT The spatial repellency responses of *Aedes aegypti* (L.) to deet, dehydrolinalool and linalool were evaluated using a dual port olfactometer. In the absence of human attractant mixture, each of the three chemicals resulted in activation and/or orientation of mosquitoes to the chemical source. Linalool was the most attractive compound. In the presence of human attractant mixture, activation and/or orientation of mosquitoes to each of the three chemicals was reduced. We compared reductions in mosquito responses to each of the three chemicals, in the presence of human attractant mixture, to estimate spatial repellency. As expected, lowest spatial repellency (7.3%) was observed using human attractant alone. Highest spatial repellency (33.6%) was observed using a combination of linalool and dehydrolinalool. Deet did not manifest spatial repellency, whereas linalool and dehydrolinalool alone, and in combination, exhibited spatial repellency.

KEY WORDS olfactometer, inhibition, linalool, dehydrolinalool, deet

TRADITIONAL APPROACHES TO MOSQUITO control and control of the disease agents they transmit are based on the use of chemical insecticides for area-wide mosquito abatement and on topical repellents for personal protection. Operational mosquito control personnel are concerned that these approaches may be severely restricted in the future. The availability of chemical insecticides has dwindled in the past two decades because of the high costs of reregistering existing compounds, the development of resistance by mosquitoes, and increased public concern about potential health and environmental hazards that result from exposure to insecticides (Kline 1994, Rathburn 1990). Deet, the most widely used topical repellent, is also under attack. But the search for replacement repellents against mosquitoes has not met with great success because most candidate topical mosquito repellents have limited effectiveness. Consequently, there is a need for new, safe, and effective ways to kill pest/vector species of mosquitoes, and to deter blood seeking mosquitoes from humans and other hosts.

The utilization of host kairomones, such as attractants and repellents, has received increased interest in the past few years as a new technology for mosquito population management (Kline 1994). Networks of attractant-baited traps have been used as a protective barrier in large areas; individual traps have been used by homeowners as killing stations in backyards (Kline and Lemire 1998). One goal of our research efforts to use kairomones for mosquito control is to combine attractant baited traps/targets with a repellent barrier. The barrier would be established by "saturating" a zone or space that contains a potential host with spatial repellent, which Gouck et al. (1967) defined as a repellent that is effective at a distance from the point of application. Nolen et al. (2002) further defined a spatial repellent as an inhibiting compound, dispensed into the atmosphere of a three dimensional environmental space, which inhibits the ability of mosquitoes to locate and track a target, such as humans or livestock. Attractant baited traps/targets, located between the source of mosquitoes and the repellent barrier, would then capture the mosquitoes.

We conduct field studies with a variety of chemical compounds, including those related to 1-octen-3-ol (octenol), in an effort to identify new and effective spatial repellents and mosquito attractants. In these studies, we have observed (D.L.K., unpublished data) that linalool (a naturally occurring volatile compound), when used alone, attracts mosquitoes to a trap; however, when used with CO_2, or with CO_2 + octenol, linalool reduces mosquito collection sizes by as much as 50%. These observations suggest that linalool acts as both an attractant and a spatial repellent. The study reported here was made to resolve this apparent disparity and to characterize the effects of linalool and related compounds on the host seeking behavior of *Aedes aegypti* (L.), in the context of spatial repellency.

Materials and Methods

A triple cage, dual-port olfactometer (Posey et al.1998), was used to evaluate the responses of female *Ae. aegypti* to candidate chemicals and to a human

This paper reports the results of research only. Mention of a chemical compound does not constitute a recommendation for use by the U.S. Department of Agriculture, nor does it imply registration under FIFRA as amended.

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attractant mixture. The olfactometer is constructed of clear acrylic and comprises three test chambers (each with two ports) in a tiered configuration. Access to the inlet port is from the front through a removable sleeve. A mosquito trap connects the inlet port to a cage holding mosquitoes. Air enters the olfactometer through a filtered external air supply system that allows precise temperature (±0.5°C) and relative humidity (±2%) control. One hour before each test, 75, 6–8 d-old female *Ae. aegypti*, are placed into a test chamber and allowed to acclimatize. A test begins once a chemical compound(s) designated for evaluation has been placed into the test port(s) and the airflow diverted over it and through the test chamber by opening a port door. Only one test chamber at a time is used.

Extensive use of the olfactometer has allowed us to characterize the behavioral patterns of mosquitoes. Typically, before the port doors are opened, most mosquitoes are at rest on the back screen or on the sides, top and bottom of the test chamber. When a candidate attractant/repellent is introduced into the test chamber most of the mosquitoes initiate flight. If the stimulus is attractive, mosquitoes orient to the source and enter the trap; often, they insert their proboscis through the screen in the direction of the attractant.

Two categories of tests, noncompetitive and competitive, were conducted. In noncompetitive tests only one of the two ports in a test chamber received a candidate attractant/repellent. These tests were made to determine if the compound(s) being tested, by itself, attracted mosquitoes. To determine a "standard" mosquito attraction response, we developed a human attractant mixture consisting of facial hair and skin (D.L.K.), removed from the shaving head of an electric razor, and acetone. We called this mixture Cara Sludge (CS). A "stock solution" of CS consisted of 619 mg of shavings placed into 85 ml of acetone. Acetone alone and in various mixtures with lactic acid has proven to be a good attractant for *Ae. aegypti* (Bernier et al. 2001). Dehydrolinalool and linalool were donated by Bedoukian Research, Inc. (Danbury, CT) and the deet was used from our stock supply (Virginia Chemicals, Inc., Portsmith, VA). Each of five concentrations (25, 100, 250, 500, and 1000 μl) of deet, dehydrolinalool, and linalool, and one concentration of CS (500 μl), were evaluated in noncompetitive tests. Chemicals were placed into the olfactometer in glass petri dishes (60 × 15 mm) that had been cleaned and sterilized. The dishes were handled using gloves to preclude contamination with skin products. At the end of each test, the number of mosquitoes that moved toward either port of a test chamber, as well as those remaining in the test chamber were counted. Mosquito responses were recorded as a percentage of the mosquitoes originally in the test chamber, that were captured in the trap, for each port. All five tests were conducted for 3 min.

In competitive tests both ports received the attractant and/or candidate repellent. Each compound to be tested was placed into one port along side of a petri dish containing 500 μl of CS. The opposite port received 500 μl CS and either an untreated petri dish or a dish containing a candidate compound. The protocol for competitive tests comprised seven comparisons of four candidate compounds (CS, deet, linalool, dehydrolinalool), in 100 μl, 250 μl, or 500 μl quantities, set in a completely randomized design. The treatment combinations were: (1) (CS versus CS), (2) (deet + CS) versus (CS), (3) (dehydrolinalool + CS) versus (CS), (4) (linalool + CS) versus (CS), (5) (deet + CS) versus (dehydrolinalool + CS), (6) (deet + CS) versus (linalool + CS), and (7) (linalool + CS) versus (dehyrdolinalool + CS). Each comparison was replicated nine times. The experimental unit was a test cage with each port (A or B) in a cage receiving one half of a treatment comparison. Cage and port-within-cage assignments were made at random within daily test times (0900, 1100, and 1300 h). Three responses were recorded for each comparison: (1) the number of mosquitoes that came to port A, (2) the number of mosquitoes that came to port B, and (3) the number of mosquitoes that were not captured (i.e., entered neither port A nor B). Responses one and two were grouped by the quantity of candidate compound tested and analyzed using student's *t* test (SAS Institute 1988). Responses for category three were pooled according to treatment combination and subjected to analysis of variance (ANOVA) with means separation using Tukey's honestly significant difference (HSD) test (SAS Institute 1988).

Results

Results from the noncompetitive tests showed that all candidate compounds caused activation and orientation of at least some mosquitoes to the source (Table 1). CS resulted in a mean attraction rate of 71.9%, which was 4.1–7.7 times more than for 500 μl of linalool, deet or dehydrolinalool. Linalool was the most attractive of the three candidate compounds tested, followed by deet and dehydrolinalool.

In the competitive tests when CS was used in both ports, 92.7% of the available mosquitoes were captured (Table 2). This compares to a capture of 71.9% in the noncompetitive tests with CS in only one port (Table 1). In every case, linalool and dehydrolinalool reduced the mean percent of mosquitoes attracted to CS (Table 2).

The results of the competitive tests (Fig. 1) illustrate the relative spatial repellency of each compound. All treatment combination compared in Fig. 1 were significantly different ($P < 0.05$), except (CS) versus (CS) and (linalool + CS) versus (dehydrolinalool + CS) at all concentrations and (dehydrolinalool + CS) versus (deet + CS) at 500 μl. These data indicate linalool and dehydrolinalool to be nearly equal as spatial repellents but better than deet, regardless of concentration. When (linalool + CS) was compared with (dehydrolinalool + CS), most mosquitoes were attracted to the dehydrolinalool port. The greatest mean difference in this regard was 11% at the 250 μl concentration. In the (linalool + CS) versus (deet +

Table 1. Results of non-competitive tests with three candidate spatial repellent compounds against *Ae. aegypti*

Compound	Concentration (μl)	Mean percent of mosquitoes (SE) collected in			
		n	Treatment port	Check port	Not collected
Deet	25	2	20.3 (3.7)	1.9 (2.7)	77.8 (4.2)
	100	3	33.6 (17.5)	1.2 (1.3)	65.2 (17.9)
	250	3	16.8 (2.6)	3.2 (2.6)	80.0 (3.3)
	500	6	15.1 (6.6)	0.6 (1.1)	84.3 (5.9)
	1000	6	19.0 (10.2)	1.1 (1.7)	79.9 (1.7)
Dehydrolinalool	25	2	18.1 (1.9)	2.5 (1.6)	79.4 (0.4)
	100	3	19.2 (8.8)	2.1 (1.4)	78.7 (9.5)
	250	3	15.9 (5.1)	4.4 (2.1)	79.7 (5.3)
	500	6	9.3 (5.7)	0.7 (1.1)	90.0 (5.1)
	1000	6	17.1 (3.1)	1.4 (1.9)	81.5 (2.4)
Linalool	25	2	37.5 (18.6)	0.8 (1.1)	61.7 (19.7)
	100	3	34.0 (15.0)	2.0 (1.4)	64.0 (14.5)
	250	3	26.1 (2.9)	3.5 (2.1)	70.4 (3.7)
	500	6	17.4 (6.7)	1.1 (1.3)	81.6 (6.6)
	1000	6	25.7 (7.6)	3.1 (2.2)	71.2 (7.9)
Cara Sludge	500	13	71.9 (10.8)	0.0 (0.0)	28.1 (10.8)

CS) tests, the deet port attracted 2.2, 1.9, and 3.2 times more mosquitoes than the linalool port at the 500, 250, and 100 ml concentrations, respectively. Similarly, in the (dehydrolinalool + CS) versus (deet + CS) tests, the deet port attracted 1.4, 2.2, and 2.9 times more mosquitoes at the 500, 250, and 100 ml concentrations, respectively, than the dehydrolinalool port.

The overall results in terms of increasing mean percent of mosquitoes captured (i.e., decreasing spatial repellency) were (CS + linalool) versus (CS + deet), (CS + dehydrolinalool) versus (CS + deet), (CS + linalool) versus (CS), (CS + dehydrolinalool) versus (CS), (CS + deet) versus (CS) and (CS) versus (CS).

Discussion

Spatial repellents can provide new technology for protection of humans and animals from mosquito transmitted disease agents. One of our research objectives is to develop spatial repellents that can be used in conjunction with attractant baited traps. The related operational goal for this objective is to use spatial repellents in the environment in a way that makes potential human or animal hosts less attractive than a nearby trap, or traps, baited with host kairomones.

Table 2. Results of competitive tests with CS and with three candidate spatial repellent compounds combined with CS

Treatment combination	Mean % (SE) not captured
(CS + linalool) × (CS + dehydrolinalool)	33.6 (1.9)a[a]
(CS + linalool) × (CS + deet)	26.6 (2.1)b
(CS + dehydrolinalool) × (CS + deet)	25.5 (2.4)b
(CS + linalool) × (CS)	24.6 (2.5)b
(CS + dehydrolinalool) × (CS)	21.1 (2.1)b
(CS + deet) × (CS)	10.3 (1.2)c
(CS) × (CS)	7.3 (1.0)c

$n = 63$.

Data combined for 100, 250, and 500 μl quantities for each treatment.

[a] Means followed by the same letter are not significantly different ($P = 0.05$) using Tukey's HSD text (SAS 1988).

Data from our tests show that linalool and dehydrolinalool manifest spatial repellency and that further evaluation of these compounds as spatial repellents is warranted. In the current study, we observed two effects of linalool and dehydrolinalool on mosquito behavior. First, in the presence of these chemicals, fewer mosquitoes in the test cage were activated to flight. Second, fewer of the activated mosquitoes were able to locate the port with the human attractant mixture, than the port without scent, and all of the mosquitoes required additional time to do so. One combination of spatial repellents was particularly noteworthy in this regard: (CS + dehydrolinalool) versus (CS + linalool). Our observations agree in principle with those of Davis and Bowen (1994) who found that individual mosquitoes, exposed to repellent alone or to repellent combined with host odor in a wind tunnel, flew more slowly than those exposed to host odor or to no host odor. They also noted that females flying upwind to host odor, turned at angles <45° to the upwind direction of air flow, whereas those flying in odor-free or repellent-laden air (either with or without host odor) made significantly more turns at angles between 45 and 90°, or >90°, to the direction of air flow.

Our findings are also consistent with those of Dogan et al. (1999) who observed that in the absence of gaseous lactic acid, deet attracted mosquitoes; on this basis, they classified deet as an inhibitor, not a repellent. Dogan et al. rationalized that an inhibitor must act in conjunction with another compound, such as lactic acid, rather than as a single compound as do repellents and attractants. Yet additional support for this hypothesis is provided by electrophysiological studies that show deet interferes with the transmission of stimuli from neurons exposed to lactic acid (Davis and Sokolove 1976, Davis 1985, Davis et al. 1987).

Field studies are needed to characterize the action of linalool and dehydrolinalool as spatial repellents against natural populations of mosquitoes. In the studies completed to date, in which linalool reduced the number of mosquitoes collected, the linalool dis-

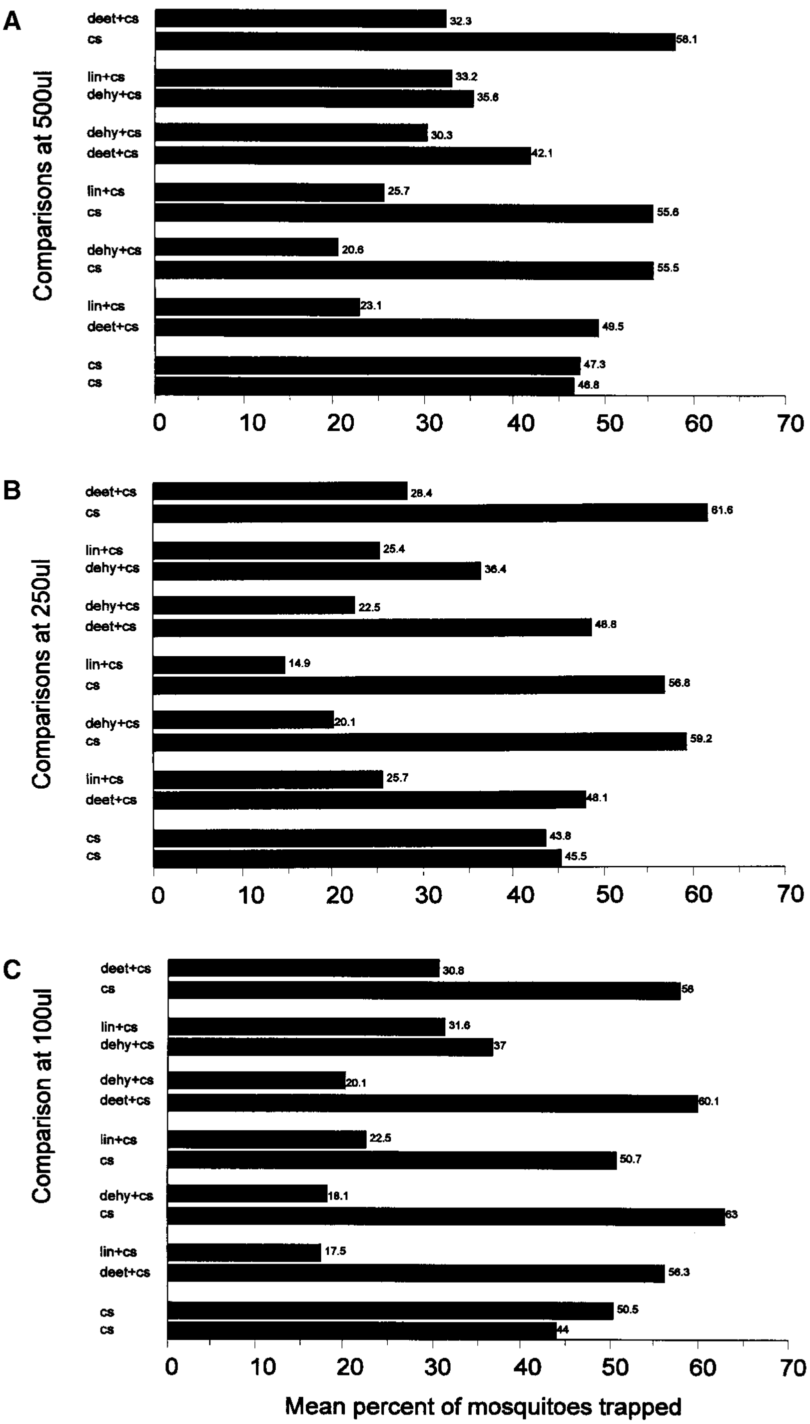

Fig. 1. (a) Results of competitive tests of CS alone (500 μl) or in combination with 100 μl deet, linalool (lin), or dehyrolinalool (dehy). (b) Results of competitive tests of CS alone (500 μl) or in combination with 250 μl deet, linalool (lin), or dehyrolinalool (dehy). (c) Results of competitive tests of CS alone (500 μl) or in combination with 500 μl deet, linalool (lin), or dehyrolinalool (dehy).

penser was mounted directly on the mosquito trap. Ideally, we would employ an independent system that delivers spatial repellent into a three dimensional space in a way that effectively masks the attractive odors produced by hosts under natural environmental conditions. Ultimately, the development of linalool, dehydrolinalool and other behavior inhibiting compounds as spatial repellents will provide an additional means of protection from mosquito attack and from infection with mosquito borne infectious agents.

Acknowledgments

The authors express their appreciation to Robert Bedoukian, Bedoukian Research, Inc., Danbury, CT, for donating the dehydrolinalool and linalool used in these tests.

References Cited

Bernier, U. R., D. L. Kline, D. R. Barnard, K. H. Posey, M. M. Booth, and R. A. Yost. 2001. Chemical compositions that attract arthropods. U. S. Patent 6,267,953.

Davis, E. E. 1985. Insect repellents: concepts of their mode of action relative to potential sensory mechanisms in mosquitoes (Diptera: Culicidae). J. Med. Entomol. 22: 237–243.

Davis, E. E., and M. F. Bowen. 1994. Sensory physiological basis for attraction in mosquitoes. J. Am. Mosq. Control Assoc. 10(2): 316–325.

Davis, E. E., and P. G. Sokolove. 1975. Temperature responses of antennal receptors of the mosquito, *Aedes aegypti.* J. Comp. Physiol. 105: 43–54.

Davis, E. E., D. A. Haggart, and M. F. Bowen. 1987. Receptors mediating host-seeking behavior in mosquitoes and their regulation by endogenous hormones. Insect Sci. Appl. 8: 637–641.

Dogan, E. B., J. W. Ayres, and P. A. Rossignol. 1999. Behavioural mode of action of deet: inhibition of lactic acid attraction. Med. Vet. Entomol. 13: 97–100.

Gouck, H. K., T. P. McGovern, and M. Beroza. 1967. Chemicals tested as space repellents against yellow-fever mosquitoes. I. Esters. J. Econ. Entomol. 60: 1587–1590.

Kline, D. L. 1994. Introduction to symposium on attractants for mosquito surveillance and control. J. Am. Mosq. Control Assoc. 10: 253–257.

Kline, D. L., and G. F. Lemire. 1998. Evaluation of attractant-baited traps/targets for mosquito management on Key Island, Florida, USA. J. of Vector Ecology. 23: 171–185.

Nolen, J. A., R. H. Bedoukian, R. E. Maloney, and D. L. Kline. 2002. Method, apparatus and compositions for inhibiting the human scent tracking ability of mosquitoes in environmentally defined three dimensional spaces. U.S. Patent No. 6,362,235. Patent issued March 26.

Posey, K. H., D. R. Barnard, and C. E. Schreck. 1998. Triple cage olfactometer for evaluating mosquito (Diptera: Culicidae) attraction responses. J. Med. Entomol. 35(3): 330–334.

Rathburn, C. B., Jr. 1990. Adulticides in the '90s: the changing picture. Wing Beats. 1: 12–13.

SAS Institute. 1988. SAS/STAT user's guide, release 6.03 edition. SAS Institute, Cary, NC.

Received for publication 14 February 2002; accepted 27 February 2003.